PHYSIOLOGICAL STUDIES ON *BOTRYOCOCCUS*. 1. EFFECT OF GROWTH PROMOTING SUBSTANCES ON THE GROWTH OF GREEN ALGA *BOTRYOCOCCUS BRAUNII**

M.R. AHMAD**

ABSTRACT – The influence of indole-3-acetic acid (IAA), tryptamine, indole and kinetin was studied on the growth of green alga *Botryococcus braunii*. Both growth promotion and inhibition were observed. Growth was promoted at 10^{-7}M IAA, 10^{-6}M tryptamine, 10^{-9}M indole and 10^{-8}M kinetin. Growth promoting effects are small in terms of biomass yield.

RÉSUMÉ – L'action de l'indole-3-acétique (IAA), de la tryptamine, de l'indole et de la kinétine sur la croissance de l'algue verte *Botryococcus braunii* a été étudiée. Une activation et une inhibition de la croissance ont été constatées. La croissance était activée à 10^{-7}M IAA, 10^{-6}M tryptamine, 10^{-9}M indole et 10^{-8}M kinétine. En terme de biomasse, les effets de l'activation de la croissance sont faibles. (traduit par la rédaction).

KEY WORDS : growth promoting substances, green alga, *Botryococcus braunii*, growth.

INTRODUCTION

Botryococcus braunii Kützing, a unicellular colonial green alga has received much attention these days due to the fact that it produces hydrocarbon and may provide a renewable source of compounds usuable as fuels or feedstocks (Largeau *et al.*, 1980; Wake & Hillen, 1980; Chirac *et al.*, 1985). The alga has a slow growth rate in nature as well as in culture conditions (Swale, 1968; Belcher, 1968) although existing evidence indicates that the photosynthetic rate in active colonies is comparable to that of other green algae (Wolf, 1983). The slow growth rate has been attributed to the hinderance of cellular gas exchange to the cells by the colonial matrix and the alga's peculiar ability to direct metabolism into metabolically expensive lipids (Belcher, 1968; Wolf, 1983). The factors responsible for slow growth are not clear but the recent data demonstrated that large improvements in growth rate and in biomass productivity can be achieved

* NBRI Research Publication No 335 (N. S.).

** Algal Biotechnology Laboratory, National Botanical Research Institute, Lucknow-226 001, India.

TABLE I

Effect of IAA, Tryptamine, Indole and Kinetin on the growth of *Botryococcus braunii*

Conc. M	IAA (A) Growth period 15 days Dry wt. mg*	IAA (B) Growth period 20 days Dry wt. mg*	Tryptamine (C) Growth period 15 days Dry wt. mg*	Indole (D) Growth period 15 days Dry wt. mg*	Kinetin (E) Growth period 15 days Dry wt. mg*
Control	18.00 ± 2.00	28.00 ± 1.41	17.00 ± 1.41	23.00 ± 2.00	17.50 ± 1.50
10^{-10}	16.00 ± 1.41	28.00 ± 1.41	19.00 ± 1.41	18.00 ± 1.41	18.50 ± 0.50
10^{-9}	14.50 ± 0.50	31.50 ± 0.50	17.50 ± 0.70	29.00 ± 1.41 (26)	17.00 ± 0.00
10^{-8}	15.00 ± 0.00	29.50 ± 0.70	17.50 ± 0.70	18.00 ± 2.00	20.50 ± 0.50 (17)
10^{-7}	14.00 ± 0.00	37.00 ± 2.00 (32)	16.50 ± 0.50	22.50 ± 0.70	16.50 ± 0.50
10^{-6}	13.50 ± 0.50	31.00 ± 1.41	21.50 ± 1.41 (26)	21.00 ± 1.41	11.50 ± 1.48
10^{-5}	10.50 ± 0.50	29.00 ± 0.00	10.00 ± 2.00	19.00 ± 1.41	12.50 ± 3.36
10^{-4}	3.50 ± 0.50	28.50 ± 0.70	7.50 ± 1.50	19.00 ± 1.41	12.50 ± 0.50
10^{-3}	2.50 ± 1.66	4.50 ± 2.06	5.00 ± 0.00	11.50 ± 1.50	5.00 ± 0.00

* Values given are means of replicates cultures (after a growth period of 15 days except (B)) with their standard deviation). Figures in parenthesis indicates the percentage increase over the control.

under appropriate «air-lift» culture conditions (Casadevall *et al.*, 1985; Brenckmann *et al.*, 1985a; Brenckmann *et al.*, 1985b.

A perusal of the literature has shown that the effect of growth promoting substances on *B. braunii* has not been studied so far. A number of growth promoting substances are known to accelerate the growth of algae (Augier, 1976, 1977). In view of this, some preliminary observations have been carried out to determine the influence of plant growth substances on the growth of *B. braunii* in culture.

MATERIAL AND METHODS

Botryococcus braunii was obtained from Texas culture collection (No 572). The alga was grown in modified Chu No. 10 medium with soil extract (5%) and M-6 trace elements. The culture was grown in 150/250 ml. «Borosil» conical flasks with 100 ml culture medium in each flask. Equal volume (10 ml) of well grown culture started from same inoculum were used as inoculum which afforded a high degree of reliability. The light (16 hr.) was provided by three fluorescent tubelight of 40 watt each (Approx. 2.700 lx.). The temperature of the culture room was maintained at 24-26°C. All aseptic precautions were taken and culture grown were rechecked for any contamination. Addition of growth promoting substances was made in the basal medium. Growth was estimated on dry weight basis. Dry weight measurement was carried out by filtration of aliquots on «Whatman» filters which were thoroughly rinsed with distilled water. The filtered algal biomass was dried at 80°C for 24 hours. Cultures were manually shaken two/three times a day.

RESULTS

Effect of indole-3-acetic acid on the growth of *B. braunii*.

The effect of varying concentrations of IAA ranging from 10^{-10} to 10^{-3} M is shown in Table I (A) after a growth period of 15 days. High concentrations (10^{-3} and 10^{-4} M) produced inhibitory effect. Lower concentrations did not stimulated growth.

After a growth period of 20 days and with slightly heavy inoculum, there is an improvement in growth relative to culture (A) as seen in table I (B). There is an increase of about 32 % in dry weight over the control at 10^{-7} M IAA treated cultures. Growth was inhibited at 10^{-3} M IAA. Growth in 10^{-4} M, 10^{-5} M, 10^{-8} M and 10^{-10} M treated cultured were similar to that of the control. There is a slight promotion of growth in 10^{-6} M and 10^{-9} M treated cultures.

Effect of potential precursors of IAA.

Tryptamine

The effect of various concentrations of tryptamine has been shown in table I (C). High concentrations (10^{-3} to 10^{-5} M) produced inhibitory effect. There is

an increase of about 26 % in dry weight over the control for 10^{-6} M tryptamine treated cultures. Growth in media containing other concentrations of tryptamine was similar to that of the control.

Indole

Growth was inhibited for 10^{-3} M indole treated cultures (table I (D)). It produced an increase of 26 % in dry weight over the control at 10^{-9} M. At other concentrations of indole, dry weight were similar to that of the control.

Effect of kinetin on *B. braunii*.

The effect of kinetin is shown in table I (E). A high concentration (10^{-3} M) produced an inhibitory effect. Other concentrations produced effects similar to the control except 10^{-8} M where there was an increase of about 17 % in dry weight over the control.

DISCUSSION

The preliminary observations of the effect of growth promoting substances has shown that there is some response. The response shown is not much in terms of biomass yield. 10^{-7} M IAA produced promotory effect only after a growth period of 20 days. At high concentration of 10^{-3} M growth was inhibited. The potential precursors of IAA showed better results on a two weeks culture. Both tryptamine and indole produced increase in growth rate at respective concentrations of 10^{-6} M and 10^{-9} M. Higher concentrations were inhibitory. Kinetin also showed some effect.

Recent studies have shown that the growth of the otherwise slow growing *B. braunii* could be accelerated by using appropriate «air-lift» systems. Casadevall *et al.* (1985) examined the growth of *B. braunii* by the above method. A mean biomass doubling time of 2.3 days was obtained during the exponential phase of batch cultures. Steady state were achieved from continuous culture with mean cell generation time of 2.77 and 2.3 days. Brenckmann *et al.* (1985a, 1985b) found great improvements using «air-lift» system of the influence of different light intensities on total biomass, hydrocarbon, cell structure, pigments and photosynthetic activity. It appears that «air-lift» conditions provides great improvements in mean generation time (2.3 days instead of one week in standard conditions). However the factors responsible for slow growth remain unclear but in view of the above observations, it is possible to enhance the growth rate using appropriate «air-lift» system, an adequately enriched growth medium with or without growth substances and perhaps mechanical agitation as well. Work on these lines are in progress.

ACKNOWLEDGEMENT

Thanks are due to the Director, National Botanical Research Institute, Lucknow for providing laboratories facilities.

REFERENCES

AUGIER H., 1976 — Les hormones des algues. État actuel des connaissances. III. Rôle des hormones dans les modalités de croissance et de développement des thalles. *Bot. Mar.* 19 : 351-377.

AUGIER H., 1977 — Les hormones des algues. État actuel des connaissances. VI. Index alphabétique par espèces des travaux sur le rôle des hormones dans la vie des algues. *Bot. Mar.* 20 : 363-379.

BELCHER J.H., 1968 — Notes on the physiology of *Botryococcus braunii* Kützing. *Arch. Mikrobiol.* 61 : 335-346.

BRENCKMANN F., LARGEAU C., CASADEVALL E. & BERKALOFF C., 1985a — Influence de la nutrition azotée sur la croissance et la production d'hydrocarbures de l'algue unicellulaire *Botryococcus braunii*. *In* PALZ W., COOMBS J. & HALL D.O. (Eds.), *Energy from biomass*. Elsevier Appl. Sci. Publ., London, pp. 717-721.

BRENCKMANN F., LARGEAU C., CASADEVALL E., CORRE B. & BERKALOFF C., 1985b — Influence of light intensity on hydrocarbon and total biomass production of *Botryococcus braunii*, relations with photosynthetic characteristics. *In* PALZ W., COOMBS J. & HALL D.O. (Eds.), *Energy from biomass*. Elsevier Appl. Sci. Publ., London, pp. 722-726.

CASADEVALL E., DIF D., LARGEAU C., GUDIN C., CHAUMONT D. & DESANTI O., 1985 — Studies on batch and continuous cultures of *Botryococcus braunii* : hydrocarbon production in relation to physiological state, cell ultrastructure, phosphate nutrition. *Biotechnol. Bioengin.* 27 : 286-295.

CHIRAC C., CASADEVALL E., LARGEAU C. & METZGER P., 1985 — Bacterial influence upon growth and hydrocarbon production of the green alga *Botryococcus braunii*. *J. Phycol.* 21 : 380-387.

LARGEAU C., CASADEVALL E., DIF D. & BERKALOFF C., 1980 — Renewable hydrocarbon production from the alga *Botryococcus braunii*. *In* PALZ W., CHARTIER P. & HALL D.O. (Ed.), *Energy from biomass*. Appl. Sci. Publi., London, pp. 653-658.

SWALE E.M.F., 1968 — The phytoplankton of Oakmere, Cheshire. *Brit. Phycol. Bull.* 3 : 441-449.

WAKE L.V. & HILLEN L.W., 1980 — Study of a bloom of the oil rich alga *Botryococcus braunii* in the Darwin river reservoir. *Biotechnol. Bioengin.* 22 : 1637-1656.

WOLF F.R., 1983 — Review : *Botryococcus braunii*, an unusual hydrocarbon producing alga. *Appl. Biochem. Biotechnol.* 8 : 249-260.